Form PTO-1390 (REV 12-29-99) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 0652.2100000/REF/PSC

U.S. APPLICATION NO. (IF KNOWN, SEE 37 C.F.R. § 1.5): To be assigned 09/601171

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/00524	27 January 1999	30 January 1998

TITLE OF INVENTION

Vaccine Formulations

APPLICANT(S) FOR DO/EO/US

Michael Buschle, Max Birnstiel and Walter Schmidt

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 372(c)(3)).
9. ☒ Facsimile copy of an oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
Preliminary Amendment and Submission of Sequence Listing;
Paper Copy of Sequence Listing (2 pages); and
Computer Readable Copy of Sequence Listing

☒ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information:
Authorization To Treat A Reply As Incorporating An Extension Of Time Under 37 C.F.R. § 1.136(a)(3)

U.S. APPLICATION NO (if known, see 37 C F R 1 50) To be assigned 09/601171	INTERNATIONAL APPLICATION NO PCT/EP99/00524	ATTORNEY'S DOCKET NUMBER 0652 2100000/REF/PSC

17. ☒ The following fees are submitted:

	CALCULATIONS PTO USE ONLY	
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$840.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$690.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... **$ 96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	- 20 =		X **$18.00**	$	
Independent Claims	- 3 =		X **$78.00**	$	
Multiple dependent claim(s) (if applicable)			+ **$260.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$	
Reduction of ½ for filing by small entity, if applicable. A Small Entity Statement must be filed. (Note 37 CFR 1.9, 1.27, 1.28)				$	
SUBTOTAL =				$ 840.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 840.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 840.00	
				Amount to be: refunded:	$
				charged:	$

a. ☒ A check in the amount of $ 840.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0036. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit Under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
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Raz E. Fleshner
SIGNATURE

Raz E. Fleshner
NAME

34,331
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Buschle *et al.*

Appl. No. To be assigned
(U.S. Natl. Phase of PCT/EP99/00524)

Filed: Herewith

For: **Vaccine Formulations**

Art Unit: To be assigned

Examiner: To be assigned

Atty. Docket: 0652.2100000/REF/PSC

Preliminary Amendment and Submission of Sequence Listing

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In advance of prosecution, please amend the application as follows:

In the Specification:

Please insert the sequence listing at the end of the application.

On page 11, line 17, after "SYDPETHI", please insert --(SEQ ID NO:1)--.

On page 12, line 7, after "LFEAIEGFI", please insert --(SEQ ID NO:2)--.

On page 12, line 7, after "GYKDGNEYI", please insert --(SEQ ID NO:3)--.

Remarks

The specification has been amended to direct the entry of this sequence listing after the claims of the above identified application and to provide corresponding SEQ ID NOs next to the sequences as they are provided in the specification.

In accordance with 37 C.F.R. § 1.821(g), this submission includes no new matter. In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above application are the same.

It is respectfully believed that this application is now in condition for examination. Early notice to this effect is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Raz E. Fleshner
Attorney for Applicants
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Date: July 28, 2000

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
Buschle *et al.*	Art Unit: To be assigned
Appl. No. To be assigned (U.S. Natl. Phase of PCT/EP99/00524)	Examiner: To be assigned
Filed: Herewith	Atty. Docket: 0652.2100000/REF/PSC
For: **Vaccine Formulations**	

Second Preliminary Amendment

Commissioner for Patents
Washington, D.C. 20231

Sir:

In advance of prosecution, please amend the application as follows.

In the Claims:

Please cancel claims 1-16.

Please add the following new claims:

--17. Vaccine containing one or more synthetic or highly purified natural peptides or proteins as antigen(s) as well as one or more adjuvants, characterised in that it is present as a solution or emulsion which is free from inorganic salt ions or has a low concentration of inorganic ions.

18. Vaccine according to claim 17, characterised in that it is substantially free from sodium and chloride and/or free from phosphate ions.

19. Vaccine according to claim 17, characterised in that it is substantially free from all inorganic salt ions.

20. Vaccine according to claim 17, characterised in that it contains one or more water-soluble or water-emulsifiable substances which are capable of making the vaccine isotonic and increasing their immunogenic activity.

21. Vaccine according to claim 20, characterised in that the isotonic-making substance is selected from the group consisting of carbohydrates, polyhydric alcohols, amino acids and lipids.

22. Vaccine according to claim 21, characterised in that the isotonic-making substance is a sugar.

23. Vaccine according to claim 21, characterised in that the isotonic-making substance is a sugar alcohol.

24. Vaccine according to claim 23, characterised in that the sugar alcohol is sorbitol.

25. Vaccine according to claim 20, characterised in that the isotonic-making substance is present in a concentration such that the resulting solution is isotonic or slightly hypotonic.

26. Vaccine according to claim 22, characterised in that the concentration of sugar is in the range from about 200-400 mM.

27. Vaccine according to claim 26, characterised in that the concentration is 250–300 mM.

28. Vaccine according to claim 23, characterised in that the concentration of sugar alcohol is in the range from about 200-400 mM.

29. Vaccine according to claim 28, characterised in that the concentration is 250–300 mM.

30. Vaccine according to claim 17, characterised in that it additionally contains a buffer.

31. Vaccine according to claim 17, characterised in that it contains a peptide as the antigen.

32. Vaccine according to claim 31, characterised in that the peptide is derived from a tumour antigen.

33. Vaccine according to claim 17, characterised in that it contains a polycation as the adjuvant.

34. Vaccine according to claim 17, characterised in that it contains polyarginine as the adjuvant.–

Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 17-34 are pending in the application, with claim 17 being the independent claim. Claims 1-16 are sought to be cancelled without

prejudice to or disclaimer of the subject matter therein. New claims 17-34 are sought to be added. Support for these new claims can be found throughout the specification and in the original claims. Specifically, support for the claim 17 can be found, *inter alia*, at page 3, lines 7-17. Support for claims 18-19 can be found, *inter alia*, at page 3, line 27 to page 4, line 11. Support for claim 20 can be found, *inter alia*, at page 4, lines 15-19. Support for claims 21-25 can be found, *inter alia,* at page 4, line 20 to page 5, line 26. Support for claims 26-29 can be found, *inter alia*, at page 5, line 27 to page 6, line 2. Support for claim 30 can be found, *inter alia*, at page 6, lines 9-17. Support for claims 31-32 can be found, *inter alia*, at page 6, lines 18-27. Support for claims 33-34 can be found, *inter alia*, at page 7, lines 7-13. These changes are believed to introduce no new matter, and their entry is respectfully requested.

It is believed that the application is now in condition for examination. Early notice to this effect is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Raz E. Fleshner
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Vaccine Formulations

The present invention relates to the field of vaccines.

The immunogenic effect of traditional vaccines is mostly based on pathogens which have been killed or attenuated. In traditional vaccines the impurities in the vaccines themselves or other components of organisms act as adjuvants which potentiate and/or prolong the immunogenic activity of the actual antigen. For example, the diphtheria-tetanus-whooping cough vaccine contains two potent adjuvants originating from the whole-cell whooping cough vaccine (LPS = lipopolysaccharide and PT = pertussis toxin); Similarly, the whole cell typhus and cholera vaccines have potent adjuvants (LPS and cholera toxin); the BCG vaccine (Bacillus Calmette Guerin) has powerful non-specific immunostimulatory effects.

In contrast to the complex traditional vaccines, modern vaccines contain synthetic, recombinant or highly purified antigens in the form of proteins or peptides. These vaccines are regarded as safer but generally have the disadvantage of lower immunogenicity. To compensate for this disadvantage, adjuvants are added to the vaccines, to increase and prolong the specific immune response to antigens. Some adjuvants have the property of intensifying T-cell proliferation and the cellular immune response.

Most of the adjuvants used hitherto have side effects, however, and furthermore these adjuvants do not meet

the requirements imposed on the safety of adjuvants, such as stability with respect to adjuvant activity, minimal toxicity with no interaction with the antigen, and also degradability in the body and the absence of any immunogenic activity of their own.

A summary of current adjuvants which have hitherto been considered for use in vaccines is provided by Vogel, 1995, and by Gupta and Siber, 1995. They include: inorganic adjuvants in gel form (aluminium hydroxide/aluminium phosphate, calcium phosphate); bacterial adjuvants such as monophosphoryl lipid A and muramyl peptides, particulate adjuvants such as the so-called ISCOMS ("immunostimulatory complexes"), liposomes and biodegradable microspheres, adjuvants based on oil emulsions and emulsifiers such as Freund's adjuvant or IFA ("Incomplete Freund's adjuvant"), saponines (such as QS-21), squalene; synthetic adjuvants such as non-ionic block copolymers, muramyl peptide analogues, synthetic lipid A, synthetic polynucleotides and polycationic adjuvants such as polyarginine or polylysine (WO 97/30721).

The choice of an adjuvant is usually a compromise which is the result of balancing the toxicity and adjuvant effect of the substance in question.

In vaccine formulations, care has generally been taken up to now to achieve isotonicity; the common vaccine formulations are usually in a salt concentration which corresponds to about 150 mM of NaCl (about 300 mosmol/l). Common buffer formulations are PBS and HBS (phosphate-buffered or HEPES-buffered saline); e.g.

for an ISCOM vaccine PBS pH 7.4 was proposed (Barr and Mitchell, 1996).

The aim of the present invention was to provide a vaccine formulation which intensifies the activity of vaccines based on antigens in the form of peptides or proteins.

It was found that, surprisingly, the immunogenic activity of a peptide-based vaccine containing adjuvant is increased if the vaccine formulation has a low concentration of salt ions or is free from salts.

The invention thus relates to a vaccine containing one or more synthetic or highly purified natural peptides or proteins as antigen(s) as well as one or more adjuvants. The vaccine is characterised in that it takes the form of a solution or emulsion which is free from inorganic salt ions or has a low concentration of salt ions.

In the context of the vaccine according to the invention the phrase "low concentration of salt ions" denotes a concentration which is equal to or less than about 50% of the salt concentration of an isotonic solution, corresponding to about 75 mM saline solution.

For calculating the ion concentration it should be borne in mind that, when using peptide or protein antigens which themselves have a charge, this charge is not taken into account.

Preferably, the vaccine is substantially free from sodium, chloride and phosphate ions, and particularly preferably it is substantially free from all inorganic

salt ions ("substantially free" means that no salts have been added to the vaccine, but that there may be impurities present which have originated from reagents or there may be traces of ions; ions originating from adjuvants are not included in the calculation either, e.g. when using inorganic adjuvants).

In the event that the vaccine contains phosphate ions, e.g. originating from buffer solution, it is preferably free from sodium and chloride ions. If it contains sodium and/or chloride ions, it is preferably free from phosphate ions.

In one embodiment of the invention the vaccine contains antigen and adjuvant in salt-free medium, e.g. in distilled water.

In another preferred embodiment the vaccine according to the invention contains one or more water-soluble or water-emulsifiable substances which are capable of making the vaccine isotonic and increasing its immunogenic activity.

These substances are hereinafter designated "isotonic-making substances". Isotonic-making substances have the property of being able to generate physiological osmotic pressure by virtue of their molecular size and molecular structure.

Preferably, the isotonic-making substances are selected from among the group carbohydrates (sugars, sugar alcohols, oligosaccharides, polysaccharides), polyhydric alcohols, amino acids or lipids.

Preferably, the isotonic-making substance is a sugar, particularly a mono- or disaccharide such as maltose, fructose, galactose or saccharose, or a sugar alcohol such as sorbitol or mannitol.

The amino acids used may be isotonic, salt-free amino acid solutions such as are used e.g. in parenteral feeding. Solutions of this kind are commercially obtainable (e.g. from Leopold, Graz, Austria); if necessary they may be desalinated if they contain salt ions. Alternatively, isotonic, salt-free solutions which contain individual, preferably water-soluble, amino acids may be used.

The lipids used may be, in particular, isotonic, salt-free fatty emulsions such as those used in parenteral feeding, for example. Emulsions of this kind are commercially obtainable (e.g. from Leopold, Graz, Austria); if necessary they may be desalinated if they contain salt ions. It is also possible to use long-chain hydrocarbons (e.g. paraffin oils), and also higher fatty acids such as linoleic acid, linolenic acid or palmitic acid, and fatty acid esters such as triglycerides.

The isotonic-making substance is preferably present in a concentration such that the resulting solution is isotonic or slightly hypotonic, depending on the molecular weight.

Preferred sugar or sugar alcohol concentrations are within the range from about 200 - 400 mM, particularly in the range from 250 - 300 mM. The osmolarity of the

solution is conveniently between 200 - 400 mosmol/l, but the solution may also be strongly hypotonic.

Amino acid solutions should preferably have an osmolarity of between 200 - 400 mosmol/l, but may also be strongly hypotonic.

Lipid emulsions also preferably have an osmolarity of between 200 - 400 mosmol/l, but may also be strongly hypotonic.

In addition to the isotonic-making substance the solution comprising the vaccine according to the invention optionally contains a buffer substance. This might be, in particular, HEPES (N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulphonic acid]), or TRIS (tris[hydroxymethyl]aminomethane). A buffer substance may be necessary to adjust the vaccine to a physiological pH if the primary solution is different from the physiological value.

The vaccine according to the invention is not subject to any restrictions regarding the peptide or protein antigens. The antigens may be naturally occurring immunogenic proteins, e.g. proteins from viral or bacterial pathogens or the fragments thereof or cellular breakdown products in the form of peptides; or tumour antigens or fragments thereof. In a preferred embodiment the antigen is a tumour antigen or a natural or synthetic peptide derived therefrom; in this case the vaccine is a tumour vaccine.

The quantity of effective antigen in the vaccine according to the invention may vary over a wide range.

The quantity of peptide depends, among other things, on the method of administration and the particular formulation used. The amount of peptide to be administered may be about 0.1 µg to about 10000 µg per vaccination dose, generally 1.0 µg to about 1000 µg, particularly about 10 µg to about 500 µg.

In a preferred embodiment of the invention the adjuvant is a substance such as that proposed in WO 97/30721, the disclosure of which is expressly referred to here, as an additive for protein or peptide vaccines, preferably a polycation such as polyarginine or polylysine which is optionally modified, e.g. with a sugar group.

The adjuvant used may also be, theoretically, any of the abovementioned adjuvants known for peptide- or protein-based vaccines. For example: inorganic adjuvants in gel form (aluminium hydroxide/aluminium phosphate, Warren et al., 1986; calcium phosphate, Relyvelt, 1986); bacterial adjuvants such as monophosphoryl lipid A (Ribi, 1984; Baker et al., 1988) and muramyl peptides (Ellouz et al., 1974; Allison and Byars, 1991; Waters et al., 1986); particulate adjuvants such as the so-called ISCOMS ("immunostimulatory complexes", Mowat and Donachie, 1991; Takahashi et al., 1990; Thapar et al., 1991), liposomes (Mbawuike et al. 1990; Abraham, 1992; Phillips and Emili, 1992; Gregoriadis, 1990) and biodegradable microspheres (Marx et al., 1993); adjuvants based on oil emulsions and emulsifiers such as Freund's adjuvant or IFA ("Incomplete Freund's adjuvant" (Stuart-Harris, 1969; Warren et al., 1986),

SAF (Allison and Byars, 1991), saponines (such as QS-21; Newman et al., 1992), squalene/squalane (Allison and Byars, 1991); synthetic adjuvants such as non-ionic block copolymers (Hunter et al., 1991), muramyl peptide analogues (Azuma, 1992), synthetic lipid A (Warren et al., 1986; Azuma, 1992), synthetic polynucleotides (Harrington et al., 1978) and polycationic adjuvants (WO 97/30721).

The skilled person will be able to define suitable antigen/adjuvant formulations from the specialist literature mentioned hereinbefore and, working from this starting point, find an isotonic-making substance which is capable of increasing the efficacy of the formulation or, while retaining the same efficacy, reducing the proportion of adjuvant in the formulation, which offers a critical advantage in the case of adjuvants with side effects.

It has surprisingly been found, within the scope of the present invention, that a salt-free tumour vaccine made isotonic with sorbitol, containing an MHC-binding peptide derived from a tumour antigen as well as polyarginine as adjuvant, has a more potent antitumour activity than a conventionally formulated tumour vaccine, i.e. containing an isotonic salt concentration, which is identical in terms of the peptide/adjuvant. It was found that the peptides together with the adjuvant dissolve better in sorbitol solution than in conventional PBS buffer. Without wishing to be tied to the theory, the improved activity of the vaccine, apart from the improved solubility, would appear to be due to the fact that the interaction

between the peptide and adjuvant is made easier and thus the activity of the adjuvant is intensified. The improved activity of the vaccine may possibly also be due to a co-adjuvant activity of the isotonic-making substance, e.g. sorbitol, i.e. this substance (sorbitol) as such has a certain adjuvant effect which increases the activity of the primary adjuvant.

The following method is appropriately used to achieve the ideal vaccine formulation: starting from a defined antigen, which is intended to provoke the desired immune response, in a first step an adjuvant matched to the antigen is found, as described in the specialist literature, particularly in WO 97/30721. In a next step the vaccine is optimised by adding various isotonic-making substances as defined in the present inventions, preferably sugars and/or sugar alcohols, in an isotonic or slightly hypotonic concentration, to the mixture of antigen and adjuvant, with the composition otherwise being identical, and adjusting the solution to a physiological pH in the range from pH 4.0 to 10.0, particularly 7.4. Then, in a first step as described in the example of the present application, the substances or the concentration thereof which will improve the solubility of the antigen/adjuvant composition compared with a conventional, saline-buffered solution are determined. The improvement in the solubility characteristics by a candidate substance is a first indication that this substance is capable of bringing about an increase in the immunogenic activity of the vaccine.

Since one of the possible prerequisites for an increase in the cellular immune response is increased binding of the antigen to APCs (antigen presenting cells), in a next step an investigation can be made to see whether the substance leads to an increase of this kind. The procedure used may be analogous to that described in the definition of the adjuvant, e.g. incubating APCs with fluorescence-labelled peptide or protein, adjuvant and isotonic-making substance. An increased uptake or binding of the peptide to APCs brought about by the substance can be determined by comparison with cells which have been mixed with peptide and adjuvant alone or with a peptide/adjuvant composition which is present in conventional saline buffer solution, using throughflow cytometry.

In a second step the candidate substances may be investigated *in vitro* to see whether and to what extent their presence is able to increase the presentation of a peptide to APCs; the MHC concentration on the cells may be measured using the methods described in WO 97/30721 for testing peptides.

Another possible way of testing the efficiency of a formulation is by using an *in vitro* model system. In this, APCs are incubated together with adjuvant, peptide and candidate substance and the relative activation of a T-cell clone which specifically recognises the peptide used is measured (Coligan et al.,1991; Lopez et al.,1993).

The efficiency of the formulation may optionally also be demonstrated by the cellular immune response by

detecting a "delayed-type hypersensitivity" (DTH) reaction in immunised animals.

Finally, the immunomodulatory activity of the formulation is measured in animal tests. In the case of a tumour vaccine as in the present example, established tumour models having known peptide sequences recognised by immune cells may be used, *inter alia*. The vaccine, containing different buffer substances but having a constant peptide/adjuvant composition, is administered to the test animals. The protection from tumour growth is a measurement of the efficacy of a tumour vaccine.

Example

The experiments were carried out as described in WO 97/30721.

a) DBA/2 mice were inoculated three times at intervals of one week with a mixture of 100 µg of MHC Class I binding peptide SYFPETHI (known as "P815 JAK1") and 75 µg of polyarginine (degree of polymerisation 70, SIGMA Chemicals, St. Louis MO) per animal. The peptide/adjuvant solution was administered in sorbitol solution (270 mM sorbitol, 5 mM HEPES) or phosphate-buffered saline solution (PBS, GIBCO BRL). Control mice were either given 100 µg of peptide/animal with no adjuvant in sorbitol buffer or were not vaccinated. A week after the last vaccination, 10^4 viable tumour cells were injected and tumour growth was measured weekly.

The results of the tests are shown in Fig. 1. The Figure shows a comparison of the efficiency of the P815 JAK1 vaccine in sorbitol solution as against a

vaccine in buffered isotonic saline solution in the animal model. It was found that animals that had been given the vaccine in sorbitol solution were better protected than mice that had been inoculated with peptide/polyarginine in PBS.

b) For the solubility tests, mixtures of fluorescence-labelled peptide LFEAIEGFI or GYKDGNEYI were prepared: 100 µg of fluorescence-labelled peptide were combined with 75 µg of polyarginine (Arg; degree of polymerisation 70, SIGMA Chemicals, St. Louis MO) either in sorbitol solution or HEPES-buffered saline solution (HBS: 20 mM HEPES pH 7.5, 150 mM NaCl). After three hours the amount of dissolved fluorescence was measured by determining the extinction at 490 nM. The test protein used was Green Fluorescent protein.

Fig. 2 and Fig. 3 show a comparison of the solubility of the complexes after mixing in buffered saline solution or sorbitol solution. The two fluorescence-labelled peptides (Fig. 2A and Fig. 2B) and the Green Fluorescent protein (GFP; about 30 Kd; Fig. 3) were included in this experiment. Adding the vaccine in sorbitol solution resulted in a significantly better solubility and recovery (increased fluorescence) both with the two tested peptides and with GFP.

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Patent Claims

1. Vaccine containing one or more synthetic or highly purified natural peptides or proteins as antigen(s) as well as one or more adjuvants, characterised in that it is present as a solution or emulsion which is free from inorganic salt ions or has a low concentration of inorganic ions.

2. Vaccine according to claim 1, characterised in that it is substantially free from sodium and chloride and/or free from phosphate ions.

3. Vaccine according to claim 1, characterised in that it is substantially free from all inorganic salt ions.

4. Vaccine according to one of the preceding claims, characterised in that it contains one or more water-soluble or water-emulsifiable substances which are capable of making the vaccine isotonic and increasing their immunogenic activity.

5. Vaccine according to claim 4, characterised in that the isotonic-making substance is selected from among the group carbohydrates, polyhydric alcohols, amino acids or lipids.

6. Vaccine according to claim 5, characterised in that the isotonic-making substance is a sugar.

7. Vaccine according to claim 5, characterised in that the isotonic-making substance is a sugar alcohol.

8. Vaccine according to claim 7, characterised in that the sugar alcohol is sorbitol.

9. Vaccine according to one of claims 4 to 8, characterised in that the isotonic-making substance is present in a concentration such that the resulting solution is isotonic or slightly hypotonic.

10. Vaccine according to claim 9, characterised in that the concentrations of sugar or sugar alcohol are in the range from about 200 - 400 mM.

11. Vaccine according to claim 10, characterised in that the concentration is 250 - 300 mM.

12. Vaccine according to one of claims 1 to 11, characterised in that it additionally contains a buffer.

13. Vaccine according to one of claims 1 to 12, characterised in that it contains a peptide as the antigen.

14. Vaccine according to claim 13, characterised in that the peptide is derived from a tumour antigen.

15. Vaccine according to one of claims 1 to 14, characterised in that it contains a polycation as the adjuvant.

16. Vaccine according to claim 15, characterised in that it contains polyarginine as the adjuvant.

Abstract

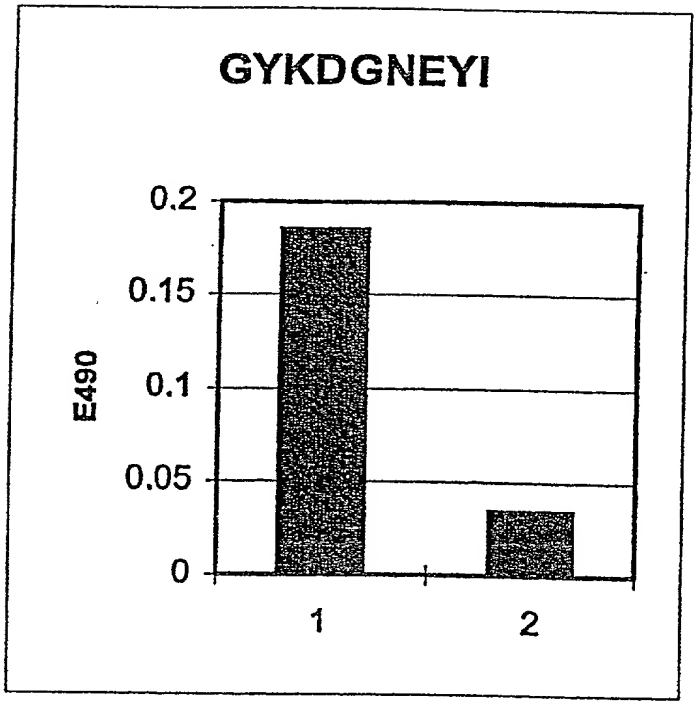
A vaccine containing one or more synthetic or highly purified natural peptides or proteins as antigen(s) as well as one or more adjuvants is present in the form of a solution or emulsion which is free from inorganic salt ions or has a low concentration of salt ions. Preferably, it contains substances capable of making the vaccine isotonic, particularly sorbitol.

09/601171

Fig. 1

Fig.2

A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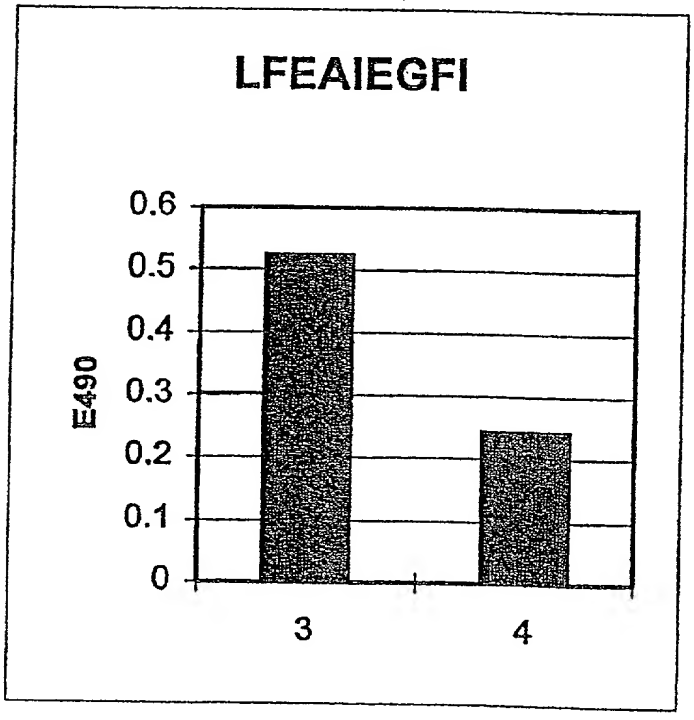
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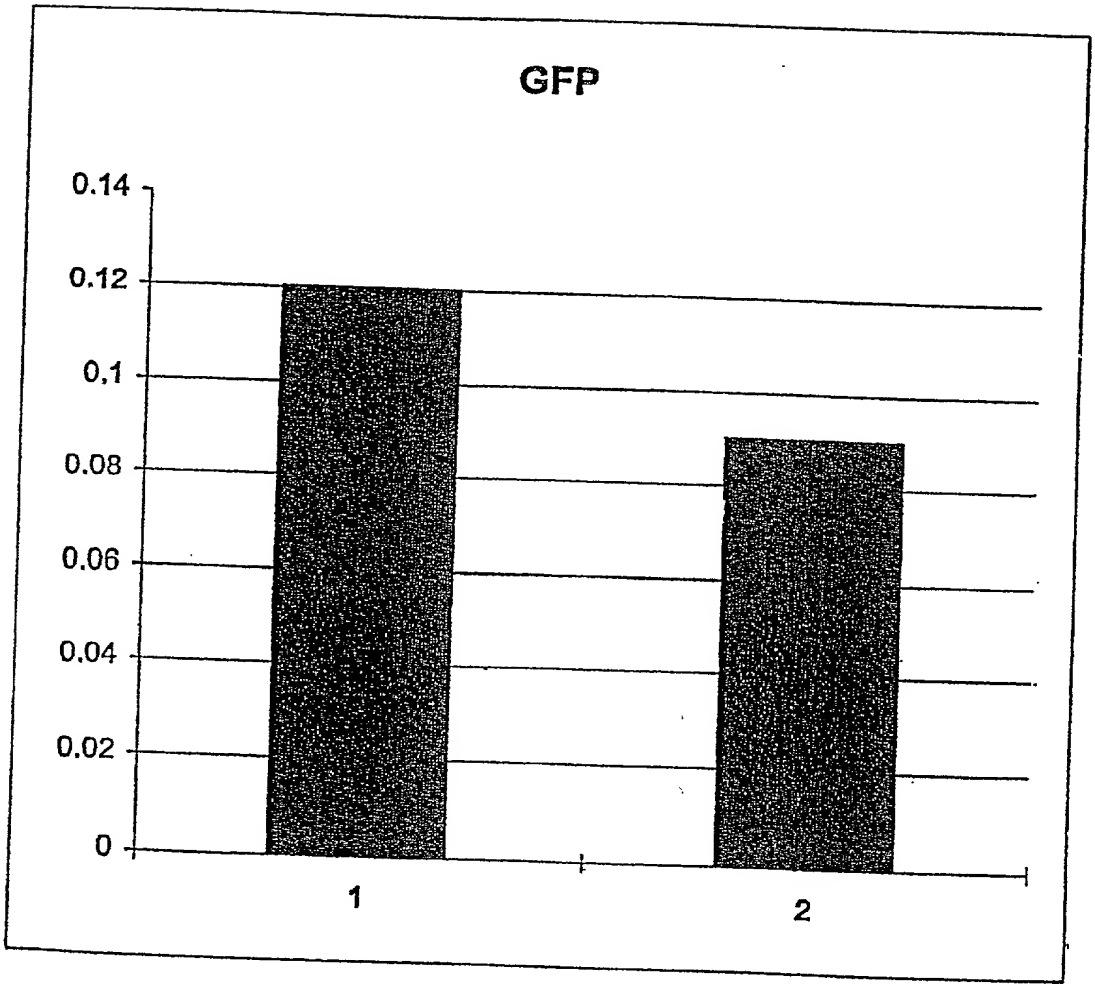
3
4

Fig. 3



Declaration for Patent Application

Appl. No. To be assigned
(U.S. Nat'l Phase of PCT/EP99/00524)
Docket Number: 0652.2100000
BI Ref. No.: 14/043-US

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled Vaccine Formulations, the specification of which is attached hereto unless the following box is checked:

☒ was filed on 27 January 1999;
as United States Application Number or PCT International Application Number PCT/EP99/00524; and
was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application, which designated at least one country other than the United States listed below, and have also identified below any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Claimed
198 03 453.9 (Application No.)	Germany (Country)	30 January 1998 (Day/Month/Year Filed)	☒ Yes ☐ No
_____ (Application No.)	_____ (Country)	_____ (Day/Month/Year Filed)	☐ Yes ☐ No

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

_____ (Application No.)	_____ (Filing Date)
_____ (Application No.)	_____ (Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56 that became available between the filing date of the prior application and the national or PCT international filing date of this application.

_____ (Application No.)	_____ (Filing Date)	_____ (Status - patented, pending, abandoned)
_____ (Application No.)	_____ (Filing Date)	_____ (Status - patented, pending, abandoned)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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(Supply similar information and signature for subsequent joint inventors, if any)